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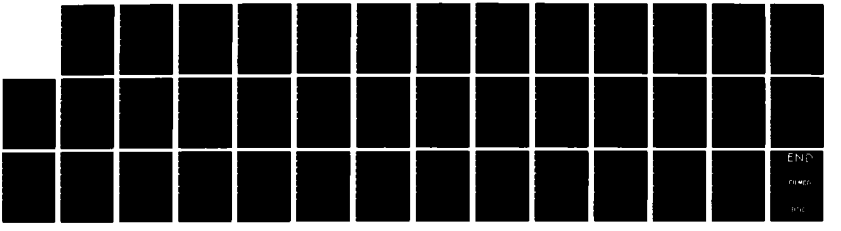

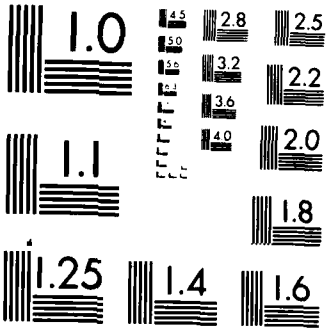

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NATIONAL RESEARCH COUNCIL

COMMISSION ON LIFE SCIENCES

2101 Constitution Avenue Washington, D.C. 20418

EXECUTIVE DIRECTOR

July 10, 1984

Dr. Eli Schmell
Code 441
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217

Dear Dr. Schmell:

In accordance with the specifications in the Office of Naval research's Contract No. N00014-83-G-0024 with the National Academy of Sciences, I am pleased to transmit to you an original and nine copies of the report Biomaterials and the U.S. Navy. This report has been prepared by the Committee on Biotechnology Applied to Naval Needs of our Commission on Life Sciences.

We are pleased to have had this opportunity to work with you and look forward to our continuing activities on behalf of ONR.

Yours sincerely,

Alvin G. Lazen, Ph.D.
Executive Director

Enclosures

NATIONAL RESEARCH COUNCIL

COMMISSION ON LIFE SCIENCES

2101 Constitution Avenue Washington, D.C. 20418

July 10, 1984

Dr. Robert W. Newburgh
Office of Naval Research
800 North Quincy Street
Arlington, Virginia 22217

Dear Dr. Newburgh:

We are pleased to transmit to you the summaries of a Conference on Biomaterials and the deliberations of our Committee on Biotechnology Applied to Naval Needs. Taken together we believe these provide a sense of the Committee's judgment on directions the Navy might consider for emphasis in its programs.

The attached summaries of the Conference were prepared by the committee members and present their interpretation of the important ideas and concepts drawn from the excellent presentations at the committee's workshop. In this letter report the Committee synthesizes that information to construct a set of general recommendations.

The Committee on Biotechnology Applied to Naval Needs was formed in the National Research Council's Commission on Life Sciences in response to a request from the U.S. Navy for advice on the application of biotechnology to meet naval needs. Initial discussions of this project included both the Naval Air Systems Command (NAVAIR) and the Biological Sciences Division of the Office of Naval Research (ONR). In subsequent discussions with ONR, which is now the sponsor of the project, it was agreed that the committee would focus on the major areas of and key developments in basic research on biomaterials without attempting to identify specific areas for development.

This report is based primarily on the proceedings of a conference on biomaterials held by the committee in Cambridge, Massachusetts, on September 19 and 20, 1983. Biomaterials are defined for the purposes of this report as substances produced by biological systems or synthesized from biologically produced subunits. The conference was divided into four major sessions: (1) Genetic Engineering, (2) Proteins and Polysaccharides, (3) Membranes and Interactions between Biological and Nonbiological Materials, and (4) The Future.

The committee believes that basic research is the limiting factor in the development of marine biotechnology. It therefore encourages ONR to continue the support of this activity in the form of support for single investigators or small groups. Development and exploitation of the results from basic research are also important, but they are proceeding successfully in the commercial sector, as well as in offices of the Department of Defense.

The committee also recommends that ONR support improvement and refinement of specific techniques. These activities may be necessary for the advancement of knowledge in the basic research areas of interest.

RESEARCH RECOMMENDATIONS

The committee has made a number of specific and general recommendations for research, but has not covered every possibility. It has not selected priorities for research in order to avoid the possibility of limiting the flexibility of the ONR in choosing potentially productive areas for its program, from among the many promising areas mentioned in the summaries or from other sources.

The Committee's recommendations fall into three categories: disciplines, techniques, and mechanisms of support.

Disciplines

The committee recommends that ONR support a variety of different research areas with special attention to the marine environment. If ONR builds bases of expertise in diverse fields, investigators associated with ONR-supported research can provide advice on specific topics relevant to naval needs and development activities. Fertile ground for advancement of biomaterials research is offered by many biological and related disciplines such as genetics, immunology, cell biology, microbiology (including procaryotes and eucaryotes as well as heterotrophs and autotrophs), biochemistry, biophysics, chemistry, and materials science.

ONR should continue its philosophy of supporting the best research projects and should encourage innovative, basic research projects independent of perceived applications. Since it is in a unique position to catalyze important advances in the marine sciences, ONR should take special responsibility for this area.

Techniques

Techniques provide the tools needed in basic research. Thus, projects involving the development and improvement of methods merit support. These projects should not be limited to the development of techniques, but should include significant basic research as well.

The following areas are among those requiring highly complex and advanced methods that merit funding: cell fusion; regeneration from cells or calluses, especially for green plants; gene cloning and recombinant DNA; generation of monoclonal antibodies and hybridomas; fermentation and separation processes; both continuous and mass culturing and harvesting of microorganisms; enzyme immobilization; protein and nucleic acid sequencing; enzyme reaction mechanisms; methods for enhancing plasmid stability; and protein synthesis. Cryobiology, cryoenzymology, and advanced instrumentation for biotechnology also merit investment by ONR.

A technical matter of great significance in marine biology is the manipulation of organisms from extreme or special environments. Of particular interest are organisms that are symbiotic, phototropic, halophilic, psychrophilic, thermophilic, acidophilic, barophilic, microaerophilic, or anaerobic. Research involving the development of methods for isolation, culture, and maintenance of such organisms is also encouraged. The culturing of apparently obligate symbionts will contribute importantly to nutritional and physiological knowledge; the culturing of organisms that are both barophilic and thermophilic is necessary if we are to gain a basic understanding of life under these conditions. In general, these kinds of research projects will lead to a fundamental understanding of the biochemical and physiological mechanisms of organisms living in extreme environments.

Mechanisms of Support

The value of and need for interdisciplinary research became obvious in discussions at committee meetings and at the Cambridge conference. Thus, the committee recommends that in addition to funding projects proposed by individual scientists, ONR should entertain proposals submitted by more than one principal investigator from diverse disciplines. University-industry cooperation should also be encouraged. Further, ONR should continue its efforts to publicize its program so as to enlarge its pool of applicants.

The committee recommends that ONR support the maintenance of marine microorganisms in the American Type Culture Collection and other such repositories. It also encourages research on the systematics of marine microorganisms to provide a data base concerning their distribution.

In summary, ONR should invest selectively in basic research, especially that which involves marine material and which may lead to the development of new methods in biotechnology. It is the committee's judgment, based in large part on the conference proceedings, that continuing to support the best scientists and the most innovative projects will offer ONR the best rewards for funding at this time.

The committee members who prepared this report and summarized the conference are listed on page iv of the attachment and the participants in the conference are listed in Appendix B. We wish to thank David Policansky of the Commission on Life Sciences staff for his able assistance and acknowledge the support of the Office of Naval Research for their support and willingness to share information with the committee.

Sincerely yours,

Rita R. Colwell

Rita R. Colwell, Ph.D.
Chairperson

BIOMATERIALS AND THE U.S. NAVY

Committee on Biotechnology Applied to Naval Needs

Commission on Life Sciences
National Research Council
Washington, D.C. 1984

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Summaries of the Conference on Biomaterials

SUMMARIES

Session 1: Genetic Engineering

This session was chaired by Anne Summers, who provided a brief overview of the very rapid developments occurring in this field. The first speaker was Ananda Chakrabarty, who reviewed work on microorganisms utilizing specific chemicals to extend their degradative capacity and described genetic approaches to problems of oil recovery and environmental pollution. Dr. Chakrabarty pointed out that hydrocarbon degradation genes are clustered on various plasmids. A number of techniques have been used to develop specific strains of bacteria that can metabolize a variety of straight-chain alkanes, such as decane, haxadecane, tetracosane, detriacontane, and other hydrocarbons. Some techniques have been developed for transposing some of these gene clusters, for molecular cloning via cosmid vectors of large fragments containing these gene clusters, and for disseminating the clusters to various bacteria under chemostatic selection with specific substrates. He described a strain of Pseudomonas aeruginosa that produces a potent emulsifier, allowing utilization of liquid hydrocarbons, such as decane, dodecane, tetradecane, or hexadecane. The strain also produces the emulsifier during growth on solid hydrocarbons, causing emulsification of the hydrocarbon in an aqueous medium. Production of these emulsifiers by P. aeruginosa, Acinetobacter sp., and other microorganisms greatly reduces the viscosity of heavy oil and helps prevent the oil from

sticking to solid surfaces. A commercial application is the emulsification of oil found in tar sands. The oil can subsequently be de-emulsified and recovered.

Biodegradation of a known persistent compound, such as 2,4,5-trichlorophenoxyacetic acid (2,4,5,-T) as a sole source of carbon and energy, was offered as another example of modification by microorganisms based on genetic and engineering methods. In this case, the strain is not only capable of utilizing 2,4,5-T as its sole carbon source, but also can completely dehalogenate a variety of halophenols such as pentachloro-, pentabromo-, or pentafluorophenol as well as a variety of tetra-, tri-, and dichlorophenols. Treatment of contaminated soil with 2,4,5-T degrading strains of Pseudomonas cepacia restores soil to a large extent so that it can support growth of plants that are sensitive to low concentrations of 2,4,5-T. Furthermore, laboratory-derived cultures can be used to remove various toxic chemicals from the environment.

Genetic engineering of marine organisms was described by Dennis Powers, who outlined a variety of areas in which genetic engineering has already been applied, notably to the control of bacterial attachment in biofouling, to modify biopolymer surfaces, to reduce or eliminate the effects of toxins, and to recover metals, among other uses. Biofouling, involves a complex series of linked events, starting with adsorption of biopolymers to surfaces and, bacterial adherence to the polymers, followed by colonization by other organisms on the conditioned surfaces. Clearly, research is needed to understand the basic biology, chemistry,

and physics of these events as well as the role of chemoreception in biofouling. One possibility for the control of biofouling would be the modification of biopolymer surfaces so that bacteria cannot "recognize" them.

New developments in marine biotechnology, especially genetic engineering, can now be applied in studies of chemoreception, and the physiology and gene regulation of marine organisms.

Dr. Powers also provided specific examples of genetic manipulation in marine fishes involving the "antifreeze gene" complex and the metallothionein gene family. These genes are regulated by changes in environmental temperature and metal concentration, respectively. The antifreeze gene can be used as a model of the ways that environmental temperatures affect functions of higher organisms. It also has a potential practical application in the storage of organs and cells at low temperature. Specifically, the antifreeze gene is involved in synthesis of a polypeptide that contains periodic saccharides. These antifreeze proteins depress the freezing point of fish fluids, but not the melting point. Both Antarctic and Arctic fishes, which continually experience low temperatures, have antifreeze genes that are always "turned on", however, fishes living in temperate water experience cold water only seasonally and need antifreeze proteins only at those times. For example, the proteins have been found only during the cold months in the winter flounder (Pseudopleuronectes americanus), which experiences temperatures as low as -1.86°C in the winter but up to 20°C during the summer. The in vivo synthesis of proteins has been correlated not

only with temperature shifts but also with changes in photoperiod. During winter and spring (November to April), few proteins other than antifreeze are synthesized. In the summer, no significant antifreeze mRNA can be detected. The appearance and disappearance of this mRNA are correlated with seasonal changes in antifreeze protein in the serum.

To analyze structure and regulation of expression of the antifreeze genes, six antifreeze proteins from the winter flounder and one from the European plaice (Pleuronectes platessa) have been sequenced. The studies show that the antifreeze proteins have a common evolutionary origin, that they have repeating segments rich in alanine, and that they comprise a gene family with varying polypeptide lengths. Their structure indicates that the polar residues are spaced in such a way that they may interrupt the ice lattice, thereby accounting for freezing point depression. Thus, the antifreeze system provides an excellent model for studying environmental parameters affecting gene expression in a marine animal and associated cellular events.

Metallothionein genes offer a mechanism for detoxification of chemical effluents, as well as recovery of valuable catalysts and detoxification of metals in chemical effluents. These are a rich source of material for research.

Dr. Powers also discussed extremely thermophilic bacteria isolated from the deep ocean thermal vents, now under study by John Baross at Oregon State University and Jody Deming at Johns Hopkins University. These investigators have shown that a mixed culture of these

microorganisms can grow within a generation as short as 40 minutes when incubated at 250°C under hydrostatic pressures of 265 atm. The bacteria reproduce entirely at the expense of inorganic sources of carbon (carbon dioxide and methane), nitrogen (ammonia and nitrate), and energy (reduced forms of sulfur, manganese, and iron). Single strains have not as yet been purified, but characteristics of the mixed cultures indicate that they have a unique genetic potential to control a variety of aerobic and anaerobic processes, including formation and utilization of oxidized and reduced metals, sulfur, trace gases, and hydrocarbons. An entirely new set of metabolic pathways may well be involved. The nucleic acids and proteins, which remain stable at temperatures previously believed to be highly denaturing, may remain functional because of the compensatory effects of elevated hydrostatic pressure. This promising area of marine microbiology merits exploration.

DNA plant technology was described as a new industry developing from recently evolved methods of protoplast fusion for plant regeneration, which may circumvent barriers of conventional hybridization and result in the production of new varieties of plants. The development of an economically feasible source of renewable fuels in North America via green plants, including several plant genera (Euphorbia, Copaifera, and Calotropis), demonstrates that there is a considerable potential for the production of commercially important hydrocarbons that may be used as fuel oils. Biotechnology methods permit improvement of both quantity and quality of hydrocarbons produced by these and other plants. Plant cell culture has been commonly used to induce somaclonal variation by taking advantage of preexisting tissue variation and mutation. In addition,

at the University of North Carolina.

Efforts should be made to construct a membrane that has a long-term stability and is sensitized with properly constructed surfactant sensitizers and to develop suitable redox catalysts for both the donor and acceptor systems. It is possible to generate hydrogen on the reductant side and oxygen on the oxidant side of a suitably constructed synthetic membrane with the catalysts already available. The development of better systems is dependent on further basic research. For example, carbon dioxide may be reduced on the reductant side, and the oxidant, instead of being lost as molecular oxygen, may be trapped as an intermediate oxidized product that may otherwise be difficult to produce.

Two lesser-known protein complexes were also discussed. One is similar to rhodopsin except that the light energy is converted to a chlorine ion gradient across the membrane, whereas the other is responsible for the repulsion or attraction of bacteria by certain wavelengths.

The direct conversion of light into energy has received much attention since the oil embargo in 1973, and it would be of great value to understand in detail how it is done in nature. Although 2% is not a great conversion efficiency, it is large enough to be interesting from an engineering point of view.

Because of the very complex structure of biological membranes, many model systems have evolved. Two limited cases exist: the lipid bilayer

resolution, the mechanism of this process is not known. In general, however, it is clear that conformational changes take place when enzymes perform their functions, but very detailed knowledge of their structure is needed to understand how their functions are performed.

Although bacterial rhodopsin and the proton pump that it operates were the only subjects involving in membrane light conversion discussed at the workshop, the much larger and different subject of photosynthesis itself should be included in the membrane research supported by ONR. Research on photosynthesis included examination not only of the natural membranes and how they work but also of the construction of totally synthetic membranes, usually in the form of vesicles. These membranes carry on their surfaces some of the surfactant photosensitizers that can induce electron transfer across such membranes. The vesicles may have an oxidized donor and reduced acceptor on either side of the membrane, and the membrane itself thus provides the barrier to the back reaction between the energy stored in the oxidant and reductant that are produced by the electron transfer. This work is under way on a substantial scale in Japan. Several laboratories in England, France, and Switzerland are also engaged in similar efforts. In the United States, studies on the subject are being conducted principally by the Department of Chemistry at the University of California, Berkeley and by Clarkson College in Potsdam, New York.

Related activities involving solid surfaces are under way in several other locations, particularly in the Department of Chemistry at the Massachusetts Institute of Technology and in the Department of Chemistry

Session 3: Membranes and Interactions Between Biological and Nonbiological Materials

Biological membranes are complex structures consisting of a bilayer of lipid molecules interspersed with protein molecules. The main function of the lipid bilayer is to separate the inside environment from the outside, while the protein molecules in the layer provide for specific functions between the two environments such as communication and transport.

Thomas Tornabene discussed the chemical composition of the lipids in some microorganisms. When cells are cultured under rapid growth conditions, the lipids make up from 1% to 24% of the dry weight of the cells, but may be as high as 80% under certain other growth conditions. In general the composition of the lipids of most bacteria is specific but rather undistinguished and relatively insensitive to various external factors. An exception to this is the membrane of the archaebacteria, which contains isoprenoid hydrocarbons and isopranyl glycerol ethers. The degree of saturation is dependent on both the aeration and the age of the culture. Dr. Tornabene commented that the degree of fatty acid saturation in both marine and freshwater algae is directly correlated to the concentration of sodium chloride in the medium.

Bacterial rhodopsin is a good example of a functioning protein in a membrane. The halobacteria derive all their energy from light absorption by this protein. The electromagnetic energy is translated into chemical energy by transport of hydrogen ions through the membrane (efficiency $\sim$2%). Even though the tertiary structure of rhodopsin is known to a 7Å

understand collagen. They prepared poly L proline, a single chain copolymer of one amino acid. They concluded that collagen may be a triple helix because a 3 amino acid polymer (proline-glycyl-proline) gave a triple helix-- and collagen itself is 3 amino acids in diameter. Furthermore, this synthetic material melts at the same temperature as collagen. Dr. Katzir tried to anneal synthetic polymers with natural collagen, but did not succeed. It would be useful to have a mixed fibrillar protein to attract or repel native proteins, because as related in the first session on genetic engineering, the availability of polyphenylalamine was of central importance to the deciphering of the genetic code. By analogy, can we use genetic engineering to make long copolymers or homopolymers?

polysaccharides there may well be other unique secondary metabolites, and these should also be looked for. All these developments and perspectives provide reason to support continued basic research in these areas.

Ephraim Katchalski-Katzir summarized and commented on the session. He pointed out that knowledge of conformation is crucial to understanding of biopolymers. Referring to Dr. Huber's discussion of proteins, including enzymes, he said it was obvious that the structure-function relationship is not yet fully understood. Nonetheless, crystallographers have made progress. Rigidity and flexibility are very important properties that are difficult to understand. He referred to Dr. Kaplan's discussion of immobilized enzymes and their use in preparing various compounds. Once again the main problem is conformational. Why are immobilized enzymes stable? Can one predict stability? The fact that an enzyme is also stereospecific is very important.

Dr. Katzir added that we have not dealt with biosensors. There will soon be a sensor to determine glucose level in the blood of diabetics. For example, there is the possibility of combining bioelectronics and physical sensors. Efforts to transfer electrons from proteins, possibly through dyes to chemical conductors such as copper wire, may be important.

Dr. Urry observed that peptides were in fashion 15 years ago, but no longer. Dr. Katzir reminded us for the sake of history that homopolymers played an important role not only in the elucidation of protein structure, including globular proteins, but also in cracking the genetic code. His own group had worked on synthetic homopolymers to try to

polysaccharides in an effort to find materials with new or improved physical characteristics.

Research in the first category has been stimulated primarily by the biological interest in the relationship of structure and function in the plant, animal, and microbial polysaccharides, especially by the emerging recognition that the poly- and oligomeric carbohydrate moieties of the glycoproteins and glycolipids found on the surfaces of eucaryotic cells play an intimate role in the immunochemistry of these cells.

Research in the second category is evidently closely linked with the first category, and it depends for its progress upon the identification of biologically active conformations and those that underlie commercially important physical properties. A comprehensive conceptual framework for polysaccharide conformational stability in the aqueous environment has yet to emerge. There is still a paucity of fundamental thermodynamic investigations of polysaccharide inter- and intramolecular conformational transformations, and the relative importance of hydrophobic, ionic, and hydrogen-bonded interactions is not yet well understood.

In the third research category, much of the current effort to discover new polysaccharide species is proprietary in nature. Recent activity has been stimulated by interest in spinning strong, highly oriented cellulosic fibers from liquid crystalline solutions and lyotropic mesophase formation by various cellulose derivatives. Several good solvents for unsubstituted cellulose and other refractory polysaccharides have recently been discovered. In addition to

Polysaccharides, a structurally diverse class of biological macromolecules, were discussed by David A. Brant. The diversity of these highly articulated linear and branched homo- and heteropolymeric structures found in nature arises from the wide array of (more than 100) carbohydrate building blocks. Polysaccharide materials from traditional plant sources (e.g., cellulose, pectin, and agarose) now may be overshadowed both in number and structural complexity by those of microbial origin. This raises the intriguing possibility that a variety of useful polymeric materials may become available at reasonable cost through large-scale microbial fermentation. Several industrially fermented microbial polysaccharides (e.g., xanthan, scleroglucan, dextran, and pullulan) have achieved commercial success. Xanthan has the remarkable ability to enhance aqueous viscosity and stabilize aqueous suspensions at very low polymer concentrations. This property has applications in the food processing and industrial coatings. Another microbial polysaccharide contains scleroglucan, a non-ionic, (1-3)-linked β-D-glucan produced in France by commercial fermentation of Sclerotium glucanium. This substance has practical application as a viscosity enhancer and as a stable gel former for suspending agents in aqueous systems.

Fundamental research on polysaccharides includes three categories: (1) investigations of the relationship of chemical structure to the conformation and the physical and biological properties of these molecules, (2) investigations of the intra- and intermolecular interactions that stabilize the biologically active and physically significant conformations, and (3) screening new microbial

The permutation of the hexamer L.Val-Gly-L.Val-L.Ala-L.Pro-Gly is a potent chemotactic peptide for elastin-synthesizing fibroblasts. Thus a synthetic polymer containing the PHP and the PPP in series in a single chain, once cross-linked, would have interesting structural and cellular effector properties for a biomaterial. In addition, the regular cross-linking sequences, which are simple alanine-rich lysine-containing sequences (designated XL), could also be introduced into the synthetic polymer to give $(PHP-XL-PPP)_n$, which would contain interesting structural and chemotactic properties and which could be expected to cross-link with the newly synthesized elastin resulting from the elastin-elaborating fibroblasts induced into the synthetic biomaterial. A more complete knowledge of these biomaterials would be of great value.

The broad area of enzymology was reviewed by Nathan O. Kaplan. During the past few years, he noted, there has been an immense increase in our understanding of the chemical, physical, and catalytic properties of enzymes. These advances have been made possible by revolutionary changes in the purification of enzymes, new procedures for carrying out specific reactions with proteins, and the novel micromethods now available for sequencing of proteins. Enzymes, even those present in the cell in very low concentrations, can be purified and studied. Techniques for immobilizing enzymes have been refined and have led to products of considerable value in understanding the properties of the biological catalysts. He also illustrated changes in properties of immobilized enzymes and described other methods modifying enzyme function. There are many important applications of immobilized enzymes in many different fields and for many different problems.

elastomeric polypeptide biomaterial can be constructed so that it can as to become covalently crosslinked by tissue enzymes to newly synthesized connective tissue protein. These perspectives are derived from sequence, conformation, and chemotactic studies on elastin peptides and from studies on enzymatic activities toward synthetic elastin peptides.

Dr. Urry noted that the precursor protein of fibrous elastin contains repeating peptide sequences. The two dominant repeat sequences are a polypentapeptide, $(L.Val\text{-}L.Pro\text{-}Gly\text{-}L.Val\text{-}Gly)_n$., and a polyhexapeptide, $(L.Ala\text{-}l.Pro\text{-}Gly\text{-}L.Val\text{-}Gly\text{-}L.Val)_n$. The value of n for the polypentapeptide (PPP) is 11 in the pig and 13 in the chick (the latter being without a single variation), and the value of n for the polyhexapeptide (PHP) is greater than five for the pig. These repeating structures have been synthesized and conformationally characterized. The high polymers have been cross-linked, the cross-linked PPP is elastomeric and capable of the same elastic modulus as the natural elastic fiber. The PHP forms cellophane-like sheets. It has been proposed that PHP forms a precross-linked aligning and interlocking role between protein chains in the fiber. Introduction of occasional lysine residues into PPP allows cross-linking to result from the activity of lysyl oxidase. Conformational studies using, in addition, cyclic analogs have substantiated the presence of a recurring secondary structural feature and have led to a new concept of elasticity referred to as a librational entropy mechanism of elasticity. Analogs in which the Gly residues have been replaced by L.Ala and D.Ala residues substantiate the new concept of elasticity and result in additional new biomaterials.

Session 2: Proteins and Polysaccharides

The second session opened with an in-depth presentation by Robert Huber, who discussed the fundamental matter of protein structure in relation to function. His analysis showed that it is still not possible to predict the higher levels of protein structure (secondary, tertiary, quaternary) from the amino acid sequence or from their functional properties. Work in the field has nevertheless been highly productive and the progress significant. We recognize structural relationships and associate certain structural features with certain functional properties, and we can be optimistic about the structural prediction of closely related families of proteins-- a much simpler problem.

Dr. Huber pointed out that proteins are often associated with nucleic acids, carbohydrates or lipids, which are essential for their function. Little is known about these interactions in structural terms, mainly because it is difficult to crystallize them. However, some new approaches appear to show promise.

In the next lecture Daniel Urry discussed new directions and current concepts in the field of elastomeric polypeptide biomaterials. He pointed out that two intriguing new perspectives have recently been derived from studies on such systems: (1) that the synthetic polypeptide biomaterial can be a direct source of chemotactic peptide for inducing cellular migration into the biomaterial and (2) that the synthetic

recombinant DNA hosts for expression of genes from terrestrial and estuarine organisms in the environment. Isolates of these bacteria may also synthesize isoenzymes that are functional at a specific range of conditions.

These discoveries lead to questions such as whether discrete gene sequences exist, one for each form of an enzyme, or whether there is transcriptional switching or posttranscriptional processing. In any case, would there be induction, modification, or processing by a protein that is itself environmentally induced? The similarity of the estuarine and abyssal systems, with respect to procaryote and invertebrate interaction, is fascinating and suggests that a fundamental principle (or principles) is involved.

In summary, the analytical strengths of molecular biology and genetics, combined with the ability to produce substrates, make it clear that genetic engineering is indeed highly attractive for application to marine systems, especially marine microbiology, for those areas appropriate to naval interests and needs.

In a summary of this session, Anne Summers remarked that the common element throughout the presentations was genetics, that is, the nature and expression of biological information. Innovations have been achieved using in vitro DNA information, adding to the power of biology as a quantitative science. The strengths of genetic engineering are in the analysis of complex functions, in the processes that underlie recruitment, and in applications to analytical biology. The panel discussants also provided interesting information. Daniel Morse commented that the possible applications of genetic engineering to the needs of the Navy arose from two major strengths of genetic engineering and related recent advances in biotechnology: the analysis of complex biological processes and the production or processing of novel or otherwise important materials.

Ronald Weiner described interaction between a procaryote and an invertebrate, i.e., a bacterium associated with the oyster, Crassostrea virginica. The unidentified bacterium, LST, produces melanin and a viscous slime layer that facilitates strong adhesion, i.e., an exopolymer. He also described a study in which an unusually large variety of marine procaryotes (Hyphomonas spp.) were recovered at 2,500 meters from a single mussel-like animal, in hydrothermal vents near the Galapagos Islands. These bacteria also synthesize adhesion polymers and melanins, and they tolerate a wide range of environments, multiplying within a pH range of 5 to 10 and in the presence of 2% to 18% salt in temperatures of $3^{\circ}C$-$50^{\circ}C$. They also grow well at atmospheric pressure, and are far more resistant to pressure changes than terrestrial procaryotes. Hyphomonas species are believed to be highly suitable

Edwin S. Lennox discussed hybridomas and monoclonal antibody production, the latter allowing work with single antibodies in an highly pure form. He pointed out that conventional antisera have long been used as specific reagents for assay and purification of a wide range of molecules, both large and small, but that they are limited because they are mixtures of large numbers of molecules with different specificities. Monoclonal antibodies have removed that limitation. Since a single antibody-forming cell produces a single antibody, cloning of these cells provides cell lines producing monoclonal antibodies. Essentially, monoclonal antibodies have two important characteristics: (1) the antibody-forming cell can be fused with a myeloma cell, thereby immortalizing it and (2) specific DNA can be cloned, thereby enabling investigators to use the DNA in an expression system. The latter technique can be generalized to antibodies of all species to allow "tailoring" of antibody properties by genetic manipulation.

Dr. Lennox pointed out that development of good <u>in vitro</u> immunization procedures for human lymphocytes has so far largely been unsuccessful and requires development. He also discussed production of rodent hybridomas and scale factors for use of monoclonal antibodies. An affinity agent monoclonal antibody can be used only to purify relatively small amounts of antigen. Therefore, these antibodies are useful as affinity reagents, but at present cannot be used to purify large volumes of materials. However, they are excellent for removing minor components, even in a fairly large volume. It is now possible to tailor antibodies for desired characteristics, and a large degree of freedom is offered in the manipulation of monoclonal antibodies.

protoplast technology enables investigators to transfer desirable genetic traits between oil-producing species and to carry out protoplast fusion and plant regeneration. Novel recombinant DNA methods are being developed to permit transfer of genes between distantly related organisms. Gene transfer via somaclonal variation, protoplast fusion, or recombinant DNA can result in the production annual crops yielding hydrocarbons or containing genes controlling characteristics involved in disease, insect, or herbicide resistance. New varieties have not yet been produced by protoplast fusion. In the future, however, it is expected that additional species will be regenerated from protoplasts, that there will be an emphasis on cytoplasmic traits, and that there will be special focus on agriculturally important crops and traits. Integration with breeding programs and interfacing with other cell culture techniques promise novel harvests.

Plant chloroplasts were mentioned as vehicles for gene transfer that offer methods for dealing with somaclonal variation, cytoplasmic male sterility, herbicide resistance, and photosynthetic efficiencies. Conference participants also discussed lyposomal fusion and molecular fingerprinting, which can provide more precise characterization of new plant varieties. Because plant cells are not like microorganisms, in that they do not excrete products, cells have to be destroyed in order to obtain the product. Therefore, since the medium for growing cells is quite expensive, ingenuity will be needed to effect secondary products synthesis on a commercial scale. Immobilization of intact plant cells or protoplasts may provide a partial answer.

without protein molecules and a protein layer without the lipids. The latter case was discussed by Sidney Fox, who described heat-aggregated amino acids, which he called protenoids. He champions the idea that protenoids were responsible for the origin of life, but the idea has not caught on in the scientific community. He made the interesting observation that heat-aggregated amino acids do not form random chains, but are at least weakly ordered. At best, only very weak enzymatic activity has been found in these proteins.

Harden McConnell described experiments with a model membrane made of lipids. To study immune response, he used liposomes that had transplantation antigens incorporated in the lipids. An even simpler membrane structure can be made simply by using one lipid film adsorbed on a solid substrate (Langmuir-Blodgett films). By this method diffusion of the lipid molecules could be studied. It was also a convenient way to study cell attachment, and again transplantation antigens can be introduced into the lipid layer for fundamental immunological studies.

Garth Nicolson gave a more medically oriented talk in which he stressed the importance of the extracellular matrix in stimulating cell development and growth. He was interested in the problem from the viewpoint of cancer as well as wound healing. By contrast with Dr. McConnell, he approached the problems from a pragmatic point of view, attempting to apply the correct sequence and amount of chemotactic factors to promote healing. The problems he has studied are presently too complex to be understood at a fundamental level.

The lesson learned from this session is that the rewards for understanding membranes and membrane functions are very large. It is clear that important advances can be made both in immunology (artificial organs, nonthrombogenic surfaces) and in wound repair. Because of the sensitivity to external stimuli, better sensors could probably be made either using biology as a model or utilizing biological materials. This is a point often discussed, but no concrete proposal for this purpose was put forward in the session. It is possible in principle to alter the membranes of cells by selection or genetic engineering, but at present we know of no such effort.

Session 4: The Future

At this session, participants integrated previous discussions on biology, chemistry, and biophysics into a materials research framework and described research trends and opportunities not particularly highlighted in other sessions.

In general, there was an endorsement of additional research on biomaterials, especially on interdisciplinary research activities. Many areas of possible research were mentioned, but no priority ranking was sought or attained. It was also clear that more substantive collaborative efforts need to be encouraged between "materials" scientists and those in other disciplines, e.g., genetics. The wide "information" and "motivation" gaps between various disciplines need to be recognized and overcome.

Although a wide array of research areas was identified, it was clear that areas not specifically mentioned were not to be considered outside the realm of interest and funding by ONR. Indeed, an open-window policy in biomaterials research may be most beneficial and realistic in terms of research management. The topics identified at the conference are discussed briefly in the following paragraphs.

Nondegradable Nucleic Acid Analogs. Substitution of the normal elements found in nucleic acids (e.g., oxygen in the phosphate bond) with other elements (e.g., carbon instead of oxygen) may provide new materials of biological significance for detection of microorganisms, delivery of

drugs, and diagnostic uses. More effective methods for attaching groups to DNA and RNA are needed.

Chemicals and specialty materials. This general category includes proteins, polysaccharides, and metabolic intermediates. Many final products, especially for medical applications, will not be those occurring naturally but, rather, will be analogs or chemically modified versions. Hence, additional research on synthetic and mechanistic chemistry is needed. Proteins include enzymes, antibodies, receptors, hormones, antigens, carriers, and storage substances.

Marine microbiology. A rational design of materials for the marine environment requires an understanding of degradation and synthetic mechanisms. In particular, an understanding of archaebacteria could be critical in sulfate reduction and corrosion inhibition or promotion. Microbial colonization of solid surfaces needs to be understood in order to control fouling, corrosion, and boundary layer rheology. Marine microorganisms should be examined for communication substances elicited by these species in response to changes in the general environment or other biological species. The biosynthesis of materials involved in cell surface adhesion should be examined. Marine luminescence needs to be more thoroughly investigated in order to use this phenomenon as a model or prototype for analytical detection systems.

Enzyme technology. The use of isolated enzymes for producing useful materials should be explored. Most of the current effort is devoted to degradation pathways, and synthetic schemes have been largely neglected

due to the complexity of the system, the sensitivity (instability) of some of the enzymes, and cofactor regeneration. Areas needing further study include the structure/property relationship of enzymes, methods for stabilizing biocatalysts, and synthetic enzymes. Isolation/purification procedures for enzymes should be improved (especially for scale-up situations), and complex multienzyme systems (some of which are contained in membranes) need to be examined and manipulated. The extracellular synthesis of complex molecules (both polymeric and asymmetric compounds) is a relatively unexplored area of research.

Fermentation and cell cultures. The stability of genetically engineered organisms in conventional and new fermentation reactors needs to be examined. In particular, microorganisms from extreme or unusual environments offer an untapped reservior of new biological materials--genes, enzymes, and other constituents--for further study and application. Animal and plant cell cultures should be examined for unique features that would make them suitable for producing specialty products (e.g., monoclonal antibodies and secondary metabolic plant products). New methods for separating low concentrations of biological compounds from a mixture of products should be developed.

Composites. Composite materials derived through the synthesis of a biologically active compound (either natural or synthetic) and a synthetic compound (usually a polymer) may exhibit some unusual properties such as stability, implantability, and time-delay characteristics, which are useful for drug delivery, food additives, and bioerodible materials. Carrier-drug conjugates may be prepared from

derivatives of drugs (e.g., catecholamines) with side-chain functional groups, which are called congeners. Conjugates with peptides and proteins including antibodies produce superactive agonists and long-lasting drugs. New selective chemical modifications need to be investigated as do the property-function relationship of composites for enhanced material properties such as mechanical strength and adhesion.

In addition to areas specifically highlighted by the speakers, ONR should consider supporting the following significant research areas:

Polymer synthesis. The synthesis of homo- or heteropolymers through enzymatic or microbial systems is an enticing endeavor. Recent advances in this area include the synthesis of cellulose fibrils by Acetobacter xylinum (Malcolm Brown, University of Texas, Austin). This bacterium produces a water-insoluble cellulose fiber that may be used in foods as well as in materials applications (such as the preparation of composites with polyesters, nylons, or polyolefins).

Immobilized enzymes. Although immobilized enzymes have been prepared and used for years, the exact nature of catalytically active species in these heterogeneous biocatalysts is not well understood. The application of modern physicochemical measurement techniques, such as nuclear magnetic resonance or electron paramagnetic resonance, could provide valuable insight into conformational and kinetic aspects of water-insoluble, fixed enzyme-polymer conjugates. Also, understanding the transfer of electrons between a coenzyme and an immobilized enzyme is a real technical limitation and needs to be investigated.

Separations. The isolation and purification of biologically produced agents is a major limitation in biotechnology. In one view, this limitation is a materials handling problem, namely, the concentration and purification of one particular compound in a mixture of similar compounds, all in extremely dilute aqueous solution. Hence, water needs to be removed in large quantity and very selectively, and specific purification procedures are needed to obtain the desired species. Generic research areas include the use of selective permeability processes, affinity chromatography, and coupled production/separation systems.

APPENDIX A

PROGRAM OF THE

CONFERENCE ON BIOMATERIALS

September 19-20, 1983

Committee on Biotechnology
Applied to Naval Needs

Commission on Life Sciences
National Research Council

Sponsored by

The United States Navy
Office of Naval Research
and
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CONFERENCE ON BIOMATERIALS
American Academy of Arts and Sciences
200 Beacon St.
Somerville, Mass.

September 19-20, 1983

SESSION 1
GENETIC ENGINEERING
Chair: ANNE SUMMERS, University of Georgia, Athens

Introduction
ANANDA CHAKRABARTY, University of Illinois, Chicago
Genetic Approaches to the Problems of Oil Recovery and Environmental Pollution
DENNIS POWERS, The Johns Hopkins University, Baltimore
Genetic Engineering of Marine Organisms
ROBERT WHITAKER, DNA Plant Technology, Inc., Cinnaminson, N.J.34
Genetic Engineering of Energy Crops
E. S. LENNOX, Medical Research Council, Cambridge
New Developments in Hybridoma Production and Usage
ANNE SUMMERS, View from the Chair

SESSION 2
PROTEINS AND POLYSACCHARIDES
Chair: EPHRAIM KATCHALSKI-KATZIR, Weizmann Institute, Rehovoth, Israel

ROBERT HUBER, Max Planck Institut, Munich, Federal Republic of Germany
Protein Structure -- The Known and the Unknown
DANIEL URRY, University of Alabama, Birmingham
Elastomeric Polypeptide Biomaterials: New Directions and Concepts
NATHAN KAPLAN, University of California, La Jolla
Developments in Enzymology
DAVID BRANT, University of California, Irvine
Polysaccharides as Biomaterials
EPHRAIM KATCHALSKI-KATZIR, View from the Chair -- Preparation and Properties of Amino Acid Polymers

SESSION 3
MEMBRANES AND INTERACTIONS BETWEEN BIOLOGICAL
AND NONBIOLOGICAL MATERIALS
Chair: JACK KYTE, University of California, La Jolla

GARTH NICOLSON, University of Texas, Houston
Extracellular Matrix-Cell Interactions, Cell Growth and Wound Repair
HARDEN MCCONNELL, Stanford University, Stanford, Calif.
Model Membrane Systems
WALTHER STOECKENIUS, University of California, San Francisco
Light Energy and Signal Transduction in Halobacteria
SIDNEY FOX, University of Miami, Coral Gables
Utilitarian Thermal Copolyamino Acids (TPAs)
THOMAS TORNABENE, Georgia Institute of Technology, Atlanta
Proximate Chemical Composition of Microorganisms with Emphasis on Lipids
JACK KYTE, View from the Chair -- Proteins and Enzymes in Biological Membranes

SESSION 4
THE FUTURE
Chair: ANTHONY SINSKEY, Massachusetts Institute of Technology, Cambridge

LESLIE ORGEL, Salk Institute, San Diego
Molecular Labels
GEORGE WHITESIDES, Harvard University, Cambridge
Biotechnology for the Production of Useful Chemicals and Materials
JOHN MARGRAVE, Rice University, Houston
New Fluorochemicals Offer Unique Solutions for Biological Problems
MURRY GOODMAN, University of California, La Jolla
Structural Aspects of Biomaterials of the Future
ANTHONY SINSKEY, View from the Chair

DISCUSSION AND CONCLUSION

APPENDIX B

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